A comparative study of nitrogen and carbon uptake by phytoplankton in a coastal eutrophic ecosystem (Bay of Brest, France)

Carbon uptake
Eutrophic ecosystem
Nitrogen uptake
Particulate matter
Phytoplankton

Absorption du carbone
Écosystème eutrophe,
Absorption de l'azote,
Matière particulaire
Phytoplancton

Sophie DAUCHEZ, Bernard QUÉGUINER, Paul TRÉGUER, Christiane ZEYONS

Laboratoire de Chimie des Écosystèmes Marins, Institut d'Études Marines, Université de Bretagne Occidentale, 6, avenue Victor Le Gorgeu, 29287 Brest Cedex.

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ABSTRACT

Nitrogen (^{15}N) and carbon (^{14}C) uptake rates and hydrological, chemical and biological parametres were monitored at a single station during spring (March-April 1989) in a coastal eutrophic ecosystem of western Europe : the Bay of Brest. Before the spring growth of phytoplankton populations, total nitrogen and carbon uptake rates were low (≈ 0.3 µmol $C.l^{-1}.h^{-1}$ and ≈ 0.025 µmol $N.l^{-1}.h^{-1}$). During the spring bloom, these uptake rates reached high values (0.5 µmol $C.l^{-1}.h^{-1}$ and 0.28 µmol $N.l^{-1}.h^{-1}$) ; at this time, nitrate was the main source of nitrogen production, although relative preference index (RPI) values showed that ammonium was the preferred nitrogen form for phytoplankton (RPI (NO_3) < 1 ; RPI (NH_4) > 5). C/N assimilation ratios were higher than C/N composition ratios of the particulate matter. This difference is interpreted in terms of terrestrial detritus inputs from rivers and variation of the physiological state of phytoplankton populations.

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RÉSUMÉ

Étude comparée de l'absorption de l'azote et du carbone par le phytoplancton dans un écosystème eutrophe (rade de Brest, France)

Un suivi des taux d'absorption d'azote (^{15}N), de carbone (^{14}C) et des paramètres hydrologiques, chimiques et biologiques a été effectué au cours du printemps (mars-avril 1989) dans un écosystème eutrophe d'Europe occidentale : la rade de Brest. En régime hivernal, les taux d'absorption du carbone et de l'azote minéral total sont faibles (≈ 0.3 µmol $C.l^{-1}.h^{-1}$ et ≈ 0.025 µmol $N.l^{-1}.h^{-1}$). Au cours du bloom printanier, les taux d'absorption atteignent des valeurs élevées (0.5 µmol $C.l^{-1}.h^{-1}$ et 0.28 µmol $N.l^{-1}.h^{-1}$) ; les nitrates représentent alors la principale source d' azote bien que les valeurs de l'indice de préférence relative (RPI) montrent une préférence du phytoplancton pour l'ammonium (RPI (NO_3) < 1 ; RPI (NH_4) > 5). L'étude des rapports d'assimilation C/N montre que ceux-ci sont supérieurs aux rapports molaires C/N de la matière particulaire. Cette différence doit être imputée aux apports fluviaux ainsi qu'aux variations de l'état physiologique des cellules phytoplanctoniques.

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INTRODUCTION

Located at the most westerly point of France, the Bay of Brest (180 km^2), here considered as a prototype of semi-enclosed coastal ecosystem (Delmas, 1981), is a shallow basin (average depth 8 m) which exchanges water with the adjacent marine ecosystem (Iroise). Waters are well mixed by tides (semi-diurnal and fortnightly periodicities); during spring tides, the tidal variation reaches 8 m, which represents an oscillating volume of 40 % of the high tide volume.

The bay is fertilized by two rivers : the Elorn in the northwest and the Aulne in the southwest ; river inputs have been estimated by Delmas (1981) in February at about 12 t $N\text{-}NO_3.d^{-1}$, 0.5 t $N\text{-}NH_4.d^{-1}$, 12 kg $P\text{-}PO_4.d^{-1}$ and 6 t $N\text{-}NO_3.d^{-1}$, 0.06 t $N\text{-}NH_4.d^{-1}$, 168 kg $P\text{-}PO_4.d^{-1}$ for the Aulne and Elorn, respectively. These natural and anthropogenic inputs exhibit large variations on both seasonal and annual scales. Thus, on an annual scale, primary production ranges from 255 to 280 g $C.m^{-2}.year^{-1}$, which classifies the Bay of Brest among eutrophic ecosystems (Quéguiner and Tréguer, 1986).

Investigations of nutrient inputs (Delmas and Tréguer, 1983), phytoplankton populations (Quéguiner and Tréguer, 1984) and phytoplankton production (Delmas *et al.*, 1983; Quéguiner and Tréguer, 1986) have been conducted in the Bay of Brest. However, although nitrogen seems to play an important role in the functioning of this ecosystem, no direct nitrogen uptake measurements have been performed so far.

Nitrogen uptake in estuarine waters has been documented in several studies, *e.g.* McCarthy *et al.* (1977) in Chesapeake Bay, Glibert *et al.* (1982) in Vineyard Sound and Carpenter and Dunham (1985) in the Carmans river estuary. These works emphasize phytoplankton preference for NH_4 relative to NO_3.

The present study was undertaken to estimate phytoplankton uptake of nitrate and ammonium, using the ^{15}N isotopic method, and to examine the relationship of the two latter parameters with carbon uptake, as measured by the ^{14}C method. Data are interpreted with reference to the hydrological and nutritional environments in the ecosystem. C/N assimilation ratios have been calculated and compared to C/N composition ratios of the particulate matter (POC/PON).

MATERIALS AND METHODS

Sampling strategy

Samples were collected twice a week at the routine station R3 (Fig. 1) considered as typical of the study area (Delmas and Tréguer, 1985). Sampling was performed during March and April 1989 at 3-m depth at 9:00 a.m. Water was collected with a 5-l Go/Flo bottle and distributed into a series of flasks.

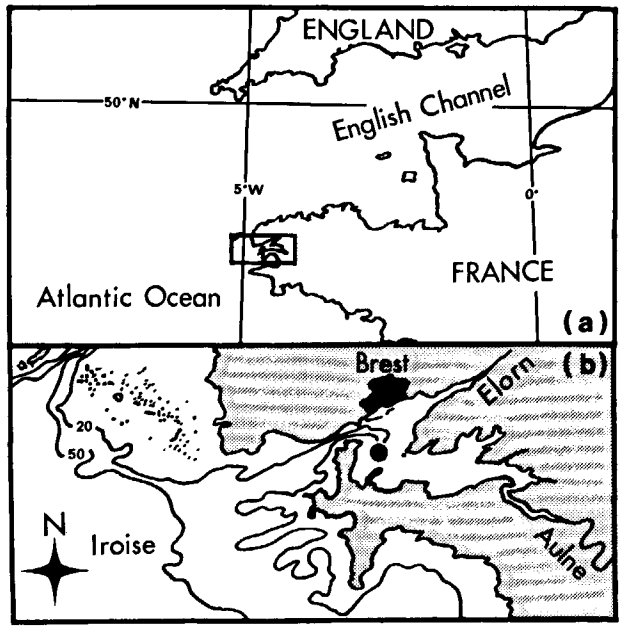

Figure 1

General situation of the study site showing location of the sampling station (o).
Localisation de la zone d'étude et de la station échantillonnée (o).

For pigments, particulate organic nitrogen (PON) and particulate organic carbon (POC) measurements, 500 ml of water were filtered onto Whatmann GF/C glass fibre filters. Phytoplankton samples were preserved with acid Lugol solution. Samples for measurement of dissolved oxygen and ammonium were fixed at once on board ship. Nutrient samples were immediately frozen, except silicate samples which were placed in a refrigerator.

Methods

Air temperature and irradiance data were obtained from the Brest-Guipavas weather station, 10 km from the study site; river flow data were obtained from the *Service Hydrologique Centralisateur* (Nantes, France). Water temperature was measured using Richter and Wiese reversed thermometers (precision ± 0.01°C); salinity was measured by the Knudsen method according to Strickland and Parsons (1972; precision ± 0.05 PSU).

Dissolved oxygen was measured by the Winkler method according to Strickland and Parsons (1972; precision ± 0.02 $ml.l^{-1}$). Ammonium measurements were made according to the manual method of Koroleff (1969; precision ± 0.05 µM). Nutrients (nitrate + nitrite, phosphate and silicate) were measured by the automated method described by Tréguer and Le Corre (1975; precision ± 0.1 µM $N\text{-}(NO_3+NO_2)$, ± 0.1 µM $Si\text{-}Si(OH)_4$, ± 0.01 µM $P\text{-}PO_4$).

Chlorophyll *a* and phaeopigments were measured using a calibrated Turner 111 fluorometer according to Hafsaoui (1984; precision ± 5 %). PON and POC measurements were performed on a modified Carlo Erba analyzer model N 1 500. Cell counts and phytoplankton species determination were made using an inverted microscope (Utermöhl, 1931).

For carbon and nitrogen uptake measurements, incubations were carried out in polycarbonate bottles over a period of four hours using non-limiting level of artificial light. The irradiance was 174 $\mu E.m^{-2}.s^{-1}$ which corresponds approximatively to surface incident radiation during the sampling period (range: 115-230 $\mu E.m^{-2}.s^{-1}$). Incubations were conducted in a constant-temperature room, at 10°C (close to the water temperature during March and April). For carbon uptake measurements, samples were incubated in 125 ml flasks (1 ml NaH $^{14}CO_3$, 148 kBq added). After incubation samples were filtered onto Whatmann GF/C glass-fibre filters. The filters were analyzed for ^{14}C uptake by liquid scintillation counting according to Quéguiner and Tréguer (1984). Nitrogen uptake rates were measured by the ^{15}N tracer technique (Dugdale and Goering, 1967), using two 500 ml flasks. In the first flask, $^{15}NO_3$ was added (≈ 10 % of ambient concentration); in the second bottle, $^{15}NH_4$ was added (≈ 20 % of ambient concentration). After incubation, samples were filtered on to precombusted Whatmann GF/C glass-fibre filters and immediately frozen until isotopic analysis. Prior to analysis, filters were dried overnight at 60°C and pounded with CaO (dessicated at 900°C for 3h) and cuprox. They were then introduced into one or several pyrex tubes according to their nitrogen content (estimated from PON measurements) in order to obtain about 6 µg nitrogen in each tube. Tubes were then sealed off and placed in a muffle furnace during 2 h at 550°C to permit deve-lopment of the oxido-reduction reaction (modified Dumas method) during which organic nitrogen is converted to N_2 gas. Isotopic ratios $^{15}N/^{14}N$ were determined by emission spectrometry (spectrometer G S 1, SOPRA) as described by Lemasson and Pages (1983).

Calculation of nitrogen uptake rates, *f*-ratios and relative preference index

Specific uptake rates (V_N as h^{-1}) were calculated using equation (a):

$$V_N = \frac{(Cp - Co)}{((Cd - Cp) \times T)} \qquad (a)$$

Transport rates (ρ_N as $\mu mol\ N\text{-}NO_3.l^{-1}.h^{-1}$ or $\mu mol\ N\text{-}NH_4.l^{-1}.h^{-1}$) were calculated using equation (b):

$$\rho_N = PONi \times V_N \qquad (b)$$

with:
- PONi : initial particulate nitrogen concentration
- Cp : concentration of the label (in atom $\%^{15}N$) in the particulate phase after incubation.
- Co: concentration of the label (in atom $\%^{15}N$) in the particulate phase at time zero.
- Cd: concentration of the label (in atom $\%^{15}N$) in the dissolved phase at time zero.
- T : incubation time.

Equation (a) and equation (b) are respectively similar to equations (4) and (7) quoted by Dugdale and Wilkerson (1986). The fact that we used PONi instead of PONf (final particulate nitrogen concentration) in our calculations could lead to an underestimation of transport rate which can be important when V_N is high. Ammonium uptake rates are not corrected for isotope dilution (Glibert *et al.*, 1982), so they are likely to have been underestimated, as Glibert *et al.* (1985) have suggested that the atom percentage enrichment of the nutrient pool (Cd) decreases exponentially during the course of incubation rather than remaining constant. In equation (b), the introduction of Cd - Cp at the denominator would usually minimize these under-estimations.

Although an underestimated transport rate could result from the use of PONi instead of PONf in equation (b), we decided to introduce it for practical reasons. Except for high V_N values, the rates are not significantly affected by this procedure. Eppley and Peterson (1979) defined the *f*-ratio as the ratio of new N/total inorganic N utilization:

$$f = \rho NO_3 / (\rho NO_3 + \rho NH_4)$$

Herein, the relative preference indices (RPI) are calculated according to McCarthy *et al.* (1977):

$$\text{For } NO_3 : RPI(NO_3) = \frac{f}{([NO_3]/[NO_3]+[NH_4])}$$

$$\text{For } NH_4 : RPI(NH_4) = \frac{1 - f}{([NH_4]/[NO_3]+[NH_4])}$$

RESULTS

Trophic environment

METEOROLOGY AND RIVER REGIMES

Throughout the study period, total irradiance tends to increase, reaching 2000 J. $cm^{-2}.d^{-1}$ at the beginning of April; air temperature exhibits low fluctuations and remains fairly constant near 10°C. River flows tend to decrease during spring, despite of some peaks.

HYDROLOGICAL AND CHEMICAL PARAMETERS

The water temperature rises slowly from the beginning

of March (10°C) to the end of April (11.5°C); salinity values frequently reaching 35 PSU appear abnormally high in comparison with the values usually measured in the Bay of Brest during the same season (range: 32-34 PSU). This is ascribed to abnormally low levels of precipitation leading to low river flows during winter 1988-1989.

During the study period, the nutrient distribution (Fig. 2) exhibits decreasing concentrations. Nitrate + nitrite concentrations decrease from 40.5 µM N-(NO_2 + NO_3) to 1.6 µM N-(NO_2+ NO_3), which is the minimum value observed at the end of April. Ammonium is characterized by low concentrations (below 0.6 µM N-NH_4) also decreasing throughout the study period. Nitrate then accounts for more than 90 % of the total inorganic nitrogen available for phytoplankton. Silicate evolution parallels that of nitrate: concentrations vary from 13.0 µM Si-Si(OH)$_4$ to 1.1 µM Si-Si(OH)$_4$ throughout March-April. Phosphates remain fairly constant in early March (about 0.50 µM P-PO_4): their concentration is regulated through adsorption-desorption processes at the water-suspended matter interface (Delmas and Tréguer, 1983). Then phosphates decrease during the phytoplankton bloom to reach the minimum value of 0.07 µM P-PO_4 on 24 April.

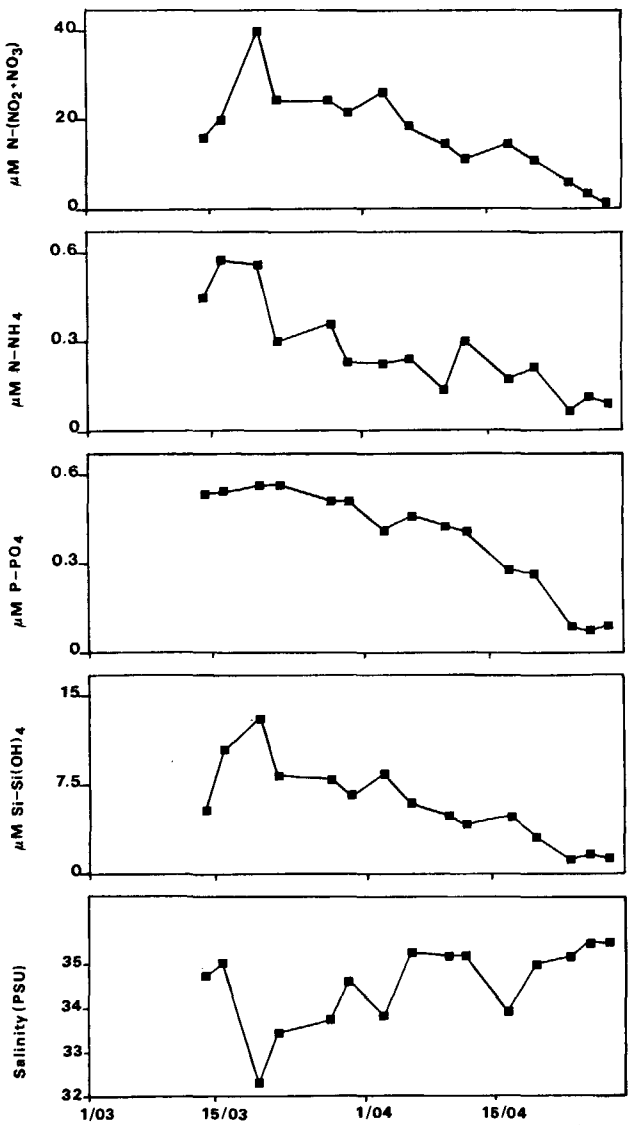

Figure 2

Temporal evolution of ambient nutrient concentrations in the Bay of Brest.
Évolution temporelle des concentrations en sels nutritifs en rade de Brest.

The overall decrease in nutrient concentration reflects utilization by phytoplankton and the decreasing contribution of river inputs.

During the study period, water remains constantly saturated with respect to dissolved oxygen (range: 102-124 % oxygen saturation).

Phytoplankton populations

During March and April 1989, microphytoplankton (linear dimension ranging between 20 to 200 µm) populations are clearly dominated by diatoms (Tab. I).

Table I

Dominant microphytoplankton species during spring (March-April 1989) in the Bay of Brest.
Espèces dominantes du microphytoplancton de la rade de Brest au cours du printemps (mars-avril 1989).

Sampling date	Dominant species
14.03	*Skeletonema costatum*
16.03	*Skeletonema costatum*
20.03	*Skeletonema costatum*
22.03	*Skeletonema costatum*
28.03	*Nitschia seriata*
30.03	*Skeletonema costatum*
03.04	*Skeletonema costatum*
06.04	*Skeletonema costatum*
10.04	*Chaetoceros curvisetum*
12.04	*Skeletonema costatum*
17.04	*Skeletonema costatum*
20.04	*Thalassiosira cf. fallax*
24.04	*Chaetoceros curvisetum*
26.04	*Chaetoceros curvisetum*
28.04	*Chaetoceros curvisetum*

Microphytoplankton and nanoplankton (linear dimension < 20 µm) exhibit different patterns of evolution (Fig. 3). As already shown by Quéguiner and Tréguer (1984) during the same season, nanoplankton cell concentrations are relatively high (from 2.2 10^5 to 6.2 10^5 cells.l^{-1}) and show few temporal fluctuations, while microphytoplankton cell concentration evolution is characterized by a succession of peaks superimposed on a general trend towards increase during spring. At the end of the study period, microphytoplankton cell concentrations remain fairly constant with high values ranging from 2 10^5 to 4 10^5 cells.l^{-1} : this coincides with the rapid and almost complete disappearance of nutrients.

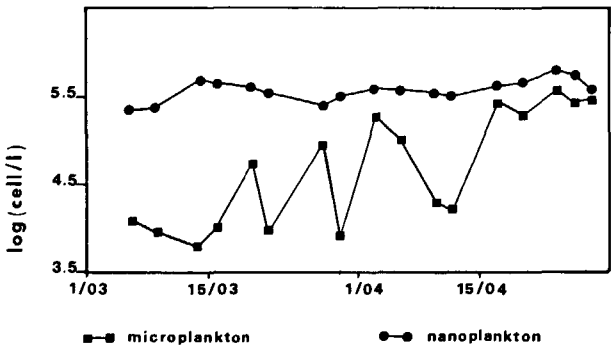

Figure 3

Temporal variation of microphytoplankton cell number and nanoplankton cell number.
Évolution temporelle des concentrations cellulaires du microphytoplancton et du nanoplancton.

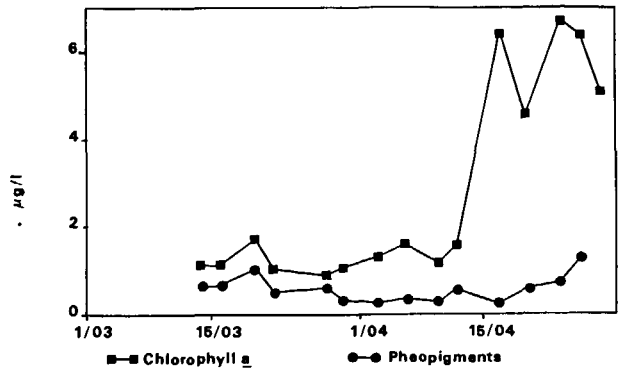

Figure 4

Temporal variation of chlorophyll a and phaeopigment concentrations.
Évolution temporelle des concentrations en chlorophylle *a* et phéopigments.

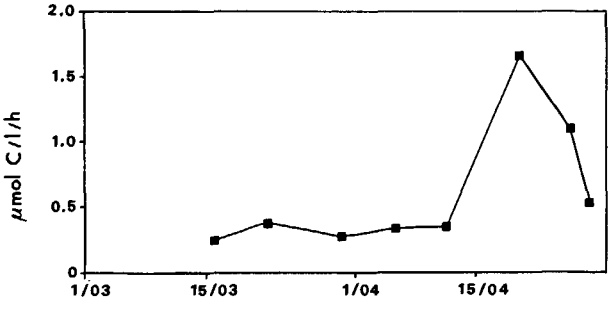

Figure 5

Temporal variation of carbon uptake rates.
Évolution temporelle des taux d'absorption de carbone.

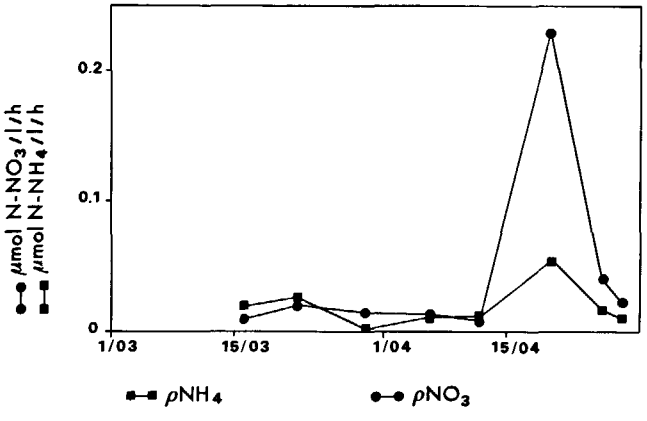

Figure 6

Temporal variation of transport rates of nitrate and ammonium.
Évolution temporelle des taux de transport des nitrates et de l'ammonium.

Carbon and nitrogen standing stocks and production

Before the beginning of the productive period, chlorophyll *a* concentrations (Fig. 4) are low (≈ 1 µg.l^{-1}); they only increase at mid-April, reaching a maximum value of 6.74 µg.l^{-1} on 24 April. Phaeopigment concentrations (Fig. 4) exhibit relatively slight variations and range between 0.22-1.23 µg.l^{-1}, the maximum value being observed at the end of the spring bloom.

PON and POC levels exhibit synchronism in fluctuations, with an average value of 10 µmol C.l^{-1} and 1.5 µmol N.l^{-1} until 12 April. After this date, both parameters increase and reach their respective maxima of 24.8 µmol C.l^{-1} and 3.8 µmol N.l^{-1} on 24 April. During March, the POC/PON molar ratio (Fig. 9) is high (≥ 7): such high values are ascribable to heavy terrigenous inputs (Tréguer and Quéguiner, 1989). During April, POC/PON molar ratios range between 6-7, *i.e.* the values are close to the average POC/PON molar ratio (6.7) as defined by Redfied *et al.* (1963). Glibert *et al.* (1982) observed a comparable evolution of POC/PON molar ratio values (from 7 to 9) for the Vineyard Sound waters.

Before spring bloom development, carbon uptake rates (Fig. 5) are low, not exceeding 0.3 µmol C.l^{-1}.h^{-1} until 12 April. By mid-April, carbon uptake rates increase suddenly to reach a maximum value of 1.7 µmol C.l^{-1}.h^{-1} on 20 April and then decrease to 0.5 µmol C.l^{-1}.h^{-1} at the end of the study period.

Nitrogen uptake parallels that of carbon. At the beginning of the study period, transport rates of ammonium (Fig. 6) are about 0.015 µmol N-NH$_4$.l^{-1}.h^{-1}. After a sharp increase, values reach a maximum (0.055 µmol N-NH$_4$.l^{-1}. h^{-1}) on 20 April.

Transport rates of nitrate (Fig. 6) show the same evolution as ammonium: values are low until mid-April, almost equal to ammonium transport rates, and then increase to reach the maximum of 0.232 µmol N-NO$_3$.l^{-1}.h^{-1} on 20 April.

As regards *f*-ratio values (Table II), these range between 0.34 and 0.94, with an average value of 0.62. During the productive period (20-28 April), *f* ranges between 0.70 and 0.80. From these values, it can be inferred that 34 to

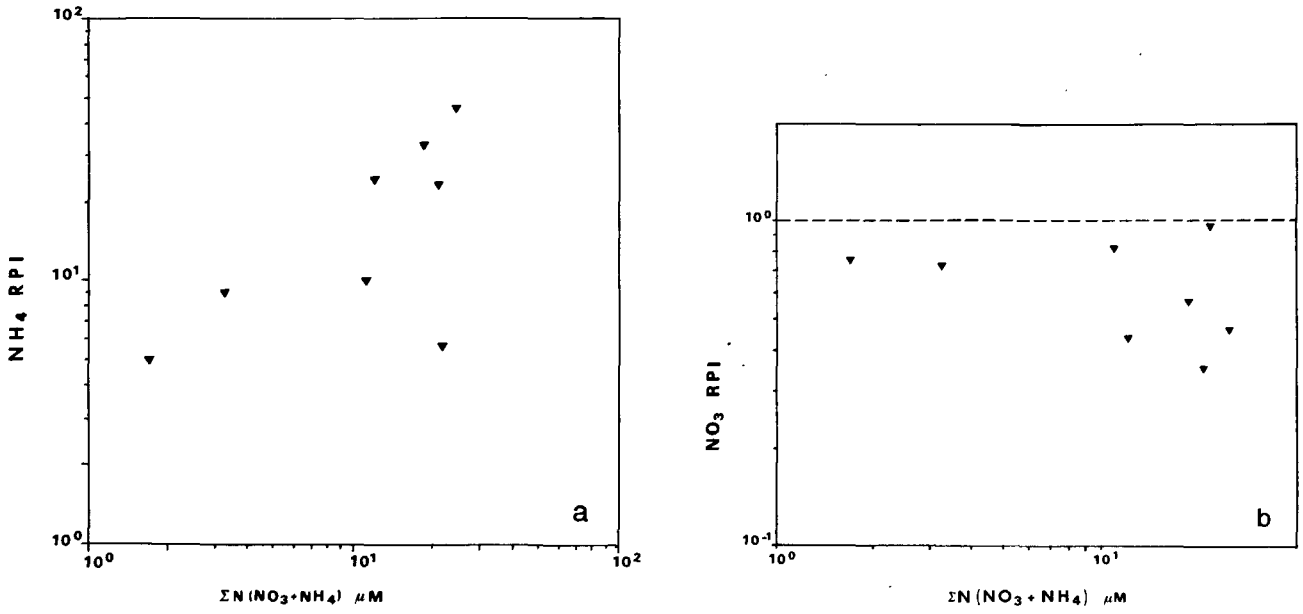

Figure 7

Evolution of RPI values [a : RPI(NH_4) ; b : RPI(NO_3)] as a function of total dissolved inorganic nitrogen concentrations.
Évolution des RPI [a : RPI(NH_4) ; b : RPI(NO_3)] en fonction des concentrations en azote minéral total dissous.

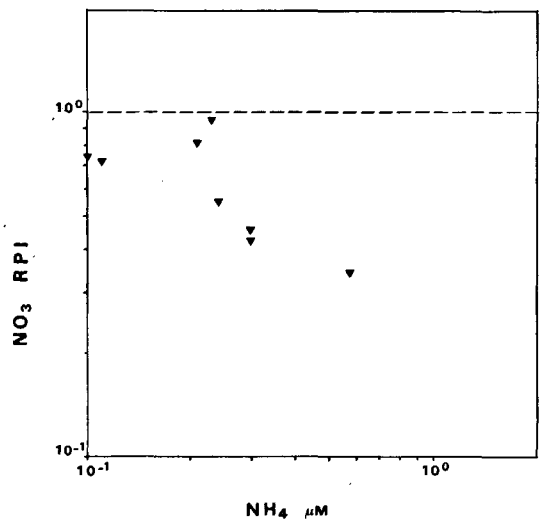

Figure 8

Evolution of RPI(NO_3) values as a function of ambient ammonium concentration.
Évolution du RPI(NO_3) en fonction des concentrations en ammonium.

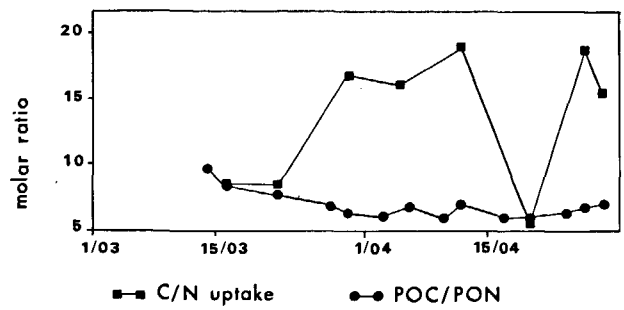

Figure 9

Temporal variation of C/N molar composition and uptake ratios of particulate matter
évolution temporelle des rapports molaires C/N de composition et d'absorption de la matière particulaire.

94 % (70-80 % during the bloom) of nitrogen production is new production.

Table II

f-ratio evolution during the study period (March-April 1989) in the Bay of Brest.
Évolution du facteur *f* en rade de Brest durant la période d'étude (mars-avril 1989).

Sampling date	*f*-ratio
16.03	0.35
22.03	0.45
30.03	0.94
06.04	0.57
12.04	0.42
20.04	0.81
26.04	0.70
28.04	0.71

RPI values for both nitrogen forms are presented as a function of total inorganic dissolved nitrogen (ΣN = nitrate + nitrite + ammonium; Fig. 7). The RPI (NO_3) values are always less than 1. No clear relationship is established between RPI (NO_3) values and ΣN. RPI (NH_4) values are always superior to unity, ranging between 5-45, and increase with increasing ΣN concentrations.

Figure 8 depicts RPI (NO_3) values as a function of ambient NH_4 concentrations. RPI (NO_3) values increase when ambient NH_4 concentrations decrease and take values superior to 0.5 when ambient NH_4 concentrations are lower than 0.3 µM N-NH_4.

C/N uptake ratios, calculated using carbon uptake and total inorganic nitrogen uptake, range between 5.8-19.1 and are generally above 10, except on 20 April when the

minimum value of 5.8 is reached (Fig. 9). C/N uptake ratios measured in the bay are generally higher than POC/PON composition ratios and almost always higher than the theoretical Redfield ratio.

DISCUSSION

Nitrogen transport rate in coastal ecosystems

Ammonium transport rates in the Bay of Brest are close to those measured in Vineyard Sound by Glibert *et al.* (1982; range: 0.01-0.10 µmol $N\text{-}NH_4.l^{-1}.h^{-1}$) and in Chesapeake Bay by Wheeler *et al.* (1982; range: 0.049-0.098 µmol $N\text{-}NH_4.l^{-1}.h^{-1}$).

On the other hand, the transport rates of nitrates we calculated exhibit a maximum much higher than that found in Vineyard Sound by Glibert *et al.* (1982; 0.02 µmol $N\text{-}NO_3.l^{-1}.\ h^{-1}$). A possible explanation of this discrepancy is that nitrate concentrations in Vineyard Sound are much more lower (range: 0.13-2.10 µM $N\text{-}NO_3$) than in the Bay of Brest (range: 1.6-40.5 µM $N\text{-}(NO_2 + NO_3)$).

According to Glibert *et al.* (1982) *f*-ratio values are close to 0.05 in oligotrophic waters, implying that 95 % of nitrogen utilized is as NH_4. For coastal waters, Eppley and Peterson (1979) report f-ratio values of 0.30-0.46.

Our *f*-ratio values are high in comparison with those reported by Eppley and Peterson (1979); they are close to values of the f_{max} calculated by Harrison *et al.* (1987) for several coastal environments. Such high values can be related to the heavy loading of new N (nitrate) in the Bay of Brest and are consistent with the positive relationship between the *f*-ratio and ambient nitrate concentration found by Platt and Harrison (1985). However we cannot preclude that N-urea uptake, not measured in the present study, might represent a significant fraction of total nitrogen uptake. Urea uptake by phytoplankton has been documented in several studies. McCarthy (1972) reported that urea uptake averaged 28 % of total nitrogen utilization in southern California coastal waters, and McCarthy *et al.* (1977) measured urea uptake rates accounting for 20.3 % of total nitrogen utilization on an annual scale in Chesapeake Bay. Moreover, Probyn and Painting (1985) found that urea uptake supply about 27 % of the nitrogen requirement for the surface water community of the Southern Ocean during the autumn. If urea uptake occurs in the same proportions in the Bay of Brest, the denominator of the *f*-ratio would be larger which would lower our calculated *f*-ratios.

According to Glibert *et al.* (1982), RPI values > 1 reflect preference for the considered nutrient while RPI values < 1 indicate selection against that nutrient. RPI (NO_3) and RPI (NH_4) values we calculated are respectively always less than and superior to unity. This means that in the Bay of Brest, during spring, ammonium is used preferentially despite the fact that nitrate accounts for more than 90 % of total inorganic nitrogen. This preference of phytoplankton for ammonium, even at high ambient nitrate concentrations, has been reported in several studies (Dugdale, 1976; Collos and Slawyk, 1980; McCarthy *et al.*, 1977; 1982; Probyn and Painting, 1985; Kristiansen and Lund, 1989). Eppley *et al.* (1969) hypothesized that phytoplankton take up ammonium preferentially because nitrate utilization requires energy for nitrate reduction, whereas ammonium assimilation does not.

Although our calculated RPI values show that phytoplankton clearly prefer ammonium, nitrate is the main source for nitrogen production in the Bay of Brest during the spring bloom. At this period, it is therefore new production which dominates although regenerated production should not be neglected (20-30 % of total nitrogen production come from ammonium utilization).

Variations of C/N uptake ratios during phytoplankton growth

C/N uptake ratios (range: 5.8-19.1) are in agreement with those of Carpenter and Dunham (1985) who found uptake ratios from 6 to 30 in the Carmans river estuary; Slawyk *et al.* (1978) reported C/N values from 5 to 45 for the northwest African upwelling area, where new production prevails.

The high uptake ratios observed at the beginning of April are related to low nitrogen-uptake rates and it can be inferred that phytoplankton have sufficient nitrogen quota.

On 20 April, when the peak of production occurs, the C/N uptake ratio suddenly decreases to a value close to the value of the POC/PON composition ratio; this is due to an enhanced nitrogen uptake rate which occurs with the increased use of intracellular nitrogen reserves during cellular divisions occurring at this time.

At the end of the study period, the C/N uptake ratio increases, which reflects the beginning of nitrogen limitation, since ambient NO_3 concentration decreased to 1.6 µM $N\text{-}NO_3$ at the end of April. Note that in eutrophic systems, values of the half-saturation constant for nitrate uptake, Kt (NO_3), are usually close to 1 µM $N\text{-}NO_3$ (McIsaac and Dugdale, 1969; Collos and Slawyk, 1980). In nitrogen-limited environments, high C/N assimilation ratios are to be expected (Carpenter and Dunham, 1985). For example, Eppley *et al.* (1977) found an average uptake ratio of 22.2 in the North Pacific gyre, and McCarthy (1972) reported a ratio of 12.4 for southern California coastal waters in which nitrogen was limiting.

Other factors may explain the high C/N uptake values we measured. Carbon and nitrogen uptake were measured simultaneously under a constant irradiance level, so we did not take account of the uncoupling between nitrogen and carbon uptake, on a per day basis, due to the duration of nitrogen uptake and the interruption of carbon fixation during the dark period (Laws and Caperon, 1976). Finally, it is not surprising to find C/N uptake ratios different from POC/PON composition ratios since uptake ratios are only indicative of the cellular metabolism of the biomass present in the water at the time of sampling whereas, in the best case (when samples contain only small amounts of detrital material), POC/PON composition ratios are the time-averaged resultant of this metabolism.

CONCLUSION

The Bay of Brest constitutes a typical coastal ecosystem of western Europe, characterized by heavy nitrogen loading originating in freshwater inputs. The spring period is characterized by a decline in major nutrients superimposed on a gradual increase in phytoplankton biomass (mainly diatoms). In such conditions, nitrogen production essentially originates from new nitrogen (evidenced from high *f*-ratios) which constitutes the main part of total dissolved inorganic nitrogen, although there is evidence of preferential uptake of ammonium against nitrate (high RPI (NH_4) values). POC/PON composition ratios exhibit great temporal variability, reflecting both detrital matter inputs from rivers and the phytoplankton elemental composition. POC/PON composition ratios reach values close to C/N uptake ratios only when C and N productions are at their maximum. C/N uptake ratios also exhibit great temporal variability, which can be interpreted in terms of variation of the nutrient status of phytoplankton during the spring bloom.

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REFERENCES

Carpenter E.J. and S. Dunham (1985). Nitrogenous nutrient uptake, primary production, and species composition of phytoplankton in the Carmans River estuary, Long Island, New York. *Limnol. Oceanogr.*, **30**, 513-526.

Collos Y. (1987). Calculations of ^{15}N uptake rates by phytoplankton assimilating one or several nitrogen sources. *Appl. Radiat. Isot.*, **38**, 275-282.

Collos Y. and G. Slawyk (1980). Nitrogen uptake and assimilation by marine phytoplancton. In: Primary productivity in the sea, P.G. Falkowski, editor, *Environ. Sci. Res.*, **19**, 195-211.

Delmas R. (1981). Étude de l'évolution saisonnière des sels nutritifs dans la rade de Brest en fonction des apports fluviaux et des échanges avec l'Iroise, *Thèse de 3°cycle, Université de Bretagne Occidentale, Brest*, 163 pp.

Delmas R. and P. Tréguer (1983). Évolution saisonnière des matières nutritives dissoutes dans un écosystème eutrophe d'Europe occidentale, *Oceanologica Acta*, **6**, 345-356.

Delmas R. and P. Tréguer (1985). Simulation de l'évolution de paramètres physiques, chimiques, et de la biomasse phytoplanctonique en période printanière dans un écosystème littoral macrotidal. *Oceanis*, 11, 197-211.

Delmas R., M. Hafsaoui, S. Le Jehan, B. Quéguiner and P. Tréguer (1983). Évolution du phytoplancton et productivité d'un écosystème eutrophe à forte variabilité saisonnière et interannuelle. Perturbations naturelles et anthropiques, *Actes du 17ème Symposium européen de Biologie Marine, Brest, 27 septembre-1er octobre 1982, Oceanol. Acta, N° sp.*, 81-85.

Dugdale R.C. and J.J. Goering (1967). Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.*, **12**, 196-206.

Dugdale R.C. (1976). Nutrient cycles. In: T*he ecology of the sea*, D.H. Cushing and J.J. Walsh, editors, Oxford, Blackwell Scientific Publication, Philadelphia, W.B. Saunders Co., 141-172.

Dugdale R.C. and F.P. Wilkerson (1986). The use of ^{15}N to measure nitrogen uptake in eutrophic oceans; experimental considerations. *Limnol. Oceanogr.*, **31**, 673-689.

Eppley R.W., J.L. Coatsworth and L. Solorzano (1969). Studies of nitrate reductase in marine phytoplankton. *Limnol. Oceanogr.*, **14**, 194-205.

Eppley R.W. and B.J. Peterson (1979). Particulate organic matter flux and planktonic new production in the deep ocean. *Nature*, **282**, 677-680.

Eppley R.W., J.H. Sharp, E.H. Renger, M.J. Perry and W.G. Harrison (1977). Nitrogen assimilation by phytoplankton and other microorganisms in the surface waters of the central North Pacific Ocean. *Mar. Biol.*, **39**, 111-120.

Glibert P.M., J.C. Goldman and E.J. Carpenter (1982). Seasonal variations in the utilization of ammonium and nitrate by phytoplankton in Vineyard Sound. *Mar. Biol.*, **70**, 237-249.

Glibert P.M., F. Lipschultz, J.J. McCarthy and M.A. Altabet (1985). Has the mystery of the vanishing ^{15}N in isotope dilution experiments been resolved?. *Limnol. Oceanogr.*, **30**, 444-447.

Grunseich G.S., R.C. Dugdale, N.F Breitner and J.J.McIsaac (1980). Sample conversion, mass spectrometry, and calculations for ^{15}N analysis of phytoplankton nutrient uptake. Coastal Upwelling Ecosystems Analysis (CUEA), Tech Rep. 44.

Hafsaoui M. (1984). Fertilisation d'un système eutrophe à forte variabilité saisonnière et annuelle (rade de Brest). Mise en évidence des facteurs limitants de la production phytoplanctonique. Assimilation simultanée des différentes formes d'azote organique et inorganique, *Thèse de 3ème cycle, Université de Bretagne Occidentale, Brest*, 167 pp.

Harrison W.G., T. Platt and M.R. Lewis (1987). *f*-Ratio and its relationship to ambient nitrate concentration in coastal waters. *J. Plankt. Res.*, **9**, 235-248.

Koroleff F. (1969). Direct determination of ammonia in natural waters as indophenol blue. *Int. Counc. Explor. Sea*, C.M., **9**, 19-22.

Kristiansen S. and B.A. Lund (1989). Nitrogen cycling in the Barents Sea. I : Uptake of nitrogen in the water column. *Deep-Sea Res.*, **36**, 2, 255-268.

Laws E. and J. Caperon (1976). Carbon and nitrogen metabolism by *Monochrysis lutheri* : measurement of growth-rate dependent respiration rates. *Mar. Biol.*, **36**, 85-97.

Lemasson L. and J. Pages (1983). Utilisation de la méthode d'analyse par spectrométrie d'émission pour la détermination de ^{15}N en écologie aquatique : assimilation du nitrate par le phytoplancton en mer de Banda (Indonésie). *J. expl. mar. Biol. Ecol.*, **67**, 33-42.

McIsaac J.J. and R.C. Dugdale (1969). The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. *Deep-Sea Res.*, **16**, 45-57.

McCarthy J.J. (1972). The uptake of urea by natural populations of marine phytoplankton. *Limnol. Oceanogr.*, 17, 738-748.

McCarthy J.J., D. Wynne and T. Berman (1982). The uptake of dissolved nitrogenous nutrients by Lake Kinneret (Israel) microplankton, *Limnol. Oceanogr.*, **27**, 673-680.

McCarthy J.J., W.R. Taylor and J.L. Taft (1977). Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1: Nutrient availability and phytoplankton preferences. *Limnol. Oceanogr.*, **22**, 996-1011.

Platt T. and W.G. Harrison (1985). Biogenic fluxes of carbon and oxygen in the ocean. *Nature*, **318**, 55-58.

Probyn T.A. and S.J. Painting (1985). Nitrogen uptake by size-fractionated phytoplankton populations in Antarctic surface waters. *Limnol. Oceanogr.*, **30**, 1327-1332.

Quéguiner B. and P. Tréguer (1984). Studies on the phytoplankton in the Bay of Brest (western Europe). Seasonal variations in composition, biomass and production in relation to hydrological and chemical features (1981-1982). *Botanica mar.*, **27**, 449-459.

Quéguiner B. and P. Tréguer (1986). Freshwater outflow effects in a coastal, macrotidal ecosystem as revealed by hydrological, chemical and biological variabilities (Bay of Brest, western Europe), In : *The role of freshwater outflow in coastal marine ecosystems*, S. Skreslet, editor, NATO ASI Series G, vol. 7, 219-230.

Redfield A.C., B.H. Ketchum and F.A. Richards (1963). The influence of organisms on composition of seawater. In: The sea, Vol. 2 M.N. Hill, editor, *Interscience*, 27-77.

Slawyk G., Y. Collos, M. Minas and J.R. Grall (1978). On the relationship between carbon-to-nitrogen composition ratios of the particulate matter and growth rate of marine phytoplankton from the northwest african upwelling area. *J. expl. mar. Biol. Ecol.*, **33**, 119-131.

Smetacek V.S. (1986). Impact of freshwater discharge on production and transfer of materials in the marine environment. In: *The role of freshwater outflow in coastal marine ecosystems*, S. Skreslet, editor, NATO ASI Series G, Vol.7, 85-106.

Strickland J.D.H. and T.R. Parsons (1972). A practical handbook of seawater analysis. *Bull. Fish. Res. Bd Can.*, **167**, 310 pp.

Tréguer P. and P. Le Corre (1975). Manuel d'analyse des sels nutritifs dans l'eau de mer. Utilisation de l'Auto-Analyzer II : Technicon. *Laboratoire d'Océanographie Chimique, Université de Bretagne Occidentale,Brest, 2ème édition*, 110 pp.

Tréguer P. and B. Quéguiner (1989). Seasonal variations in conservative and non conservative mixing of nitrogen compounds, in a West European macrotidal estuary. *Oceanologica Acta.* **12**, 371-380.

Utermöhl M. (1931). Über das umgekehrte Mikroskop. *Arch. Hydrobiol., Beih., Ergebn. Plankt.*, **22**, 643-645

Wheeler P.A., P.M. Glibert, J.J. McCarthy (1982). Ammonium uptake and incorporation by Chesapeake Bay phytoplankton : short term uptake kinetics. *Limnol. Oceanogr.*, **27**, 1113-1128.